WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
Coden USA: WJPRAP Impact Factor 8.63
Volume 15, Issue 18, 559-569. Research Article ISSN 2277–7105

IN VITRO ANTIBACTERIAL ACTIVITY AND MINIMUM INHIBITORY CONCENTRATION OF METHANOLIC EXTRACT OF *MESUA FERREA* L. FLOWERS AGAINST PATHOGENIC BACTERIA

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Article Received on 12 August 2026,
Article Revised on 02 Sept. 2026,
Article Published on 16 Sept. 2026,

https://doi.org/10.5281/zenodo.22766632

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How to cite this Article: A. Rajasekaran*, B. Akshya (2026). In Vitro Antibacterial Activity And Minimum Inhibitory Concentration Of Methanolic Extract Of Mesua Ferrea L. Flowers Against Pathogenic Bacteria. World Journal of Pharmaceutical Research, 15(18), 559–569.

ABSTRACT

Background: *Mesua ferrea* L. flowers have a history of use in traditional medicine and have been investigated for antimicrobial activity. Previous studies on different parts and extracts of the plant have reported activity against a range of Gram-positive and Gram-negative organisms. The present study evaluated the *in vitro* antibacterial activity of a methanolic extract of *M.ferrea* flower buds against ten bacterial strains representing both Gram-positive and Gram-negative organisms. **Methods:** Dried *M. ferrea* flower buds were extracted by cold maceration with methanol. Antibacterial activity was assessed by agar well diffusion, and the minimum inhibitory concentration (MIC) was evaluated by broth microdilution using two-fold serial concentrations from 1000 to 7.81 µg/mL. The bacterial strains and culture collection numbers were documented. **Results:** The methanolic extract produced inhibition zones against nine of the ten tested strains, with zones ranging from 16 to 28 mm; no inhibition was recorded for *Bacillus subtilis*. The largest inhibition zone was observed against *Escherichia coli* (28 mm), followed by *Staphylococcus aureus* (27 mm). MIC values ranged from 62.5 to 250 µg/mL. The lowest MIC (62.5 µg/mL) was observed against *Pseudomonas aeruginosa* and *Micrococcus luteus*, whereas the remaining susceptible strains showed MIC values of 125 or 250 µg/mL. **Conclusion:** The findings demonstrate measurable broad-spectrum antibacterial activity of the methanolic extract of *M.ferrea* flower buds, although the response varied among organisms and the tested organism panel represents only part of the broader antibacterial spectrum reported for this species in the

literature. The present dataset supports further investigation of the extract and its bioactive constituents.

KEYWORDS: *Mesua ferrea*; flower buds; methanolic extract; antibacterial activity; agar well diffusion; minimum inhibitory concentration; MIC.

1. INTRODUCTION

Mesua ferrea L., commonly known as Nagkesar, is a flowering tree belonging to the family Clusiaceae and is widely distributed in South and Southeast Asia. Different parts of the plant, including flowers, seeds and stamens, have been traditionally used in Siddha and Ayurvedic medicine for various purposes, including antimicrobial applications. Previous investigations have reported antimicrobial activity of *M. ferrea* extracts against different bacterial organisms.

Mesua ferrea extracts from various plant parts have been reported to possess antibacterial activity against a range of organisms (Table 1), but these reports differ considerably in plant part used, extraction solvent, and organism panel tested. Where a concentration is reported, it typically reflects a single tested concentration at which activity was observed, rather than a minimum inhibitory concentration determined through a serial dilution series. Consequently, the existing literature does not offer a reproducible, quantitative antibacterial profile for methanolic extracts of *M. ferrea* flower buds evaluated against a defined, mixed Gram-positive/Gram-negative panel using both zone-of-inhibition and MIC endpoints. The present study addresses this gap by determining MIC values across a two-fold dilution series (7.81–1000 µg/mL), thereby providing a quantitative measure of comparative susceptibility that has not previously been established for this combination of plant material and organism panel. Accordingly, the present work was undertaken to evaluate the antibacterial activity of a methanolic extract of *M. ferrea* flower buds against ten bacterial strains, comprising five Gram-positive and five Gram-negative organisms, using agar well diffusion and broth microdilution, with the specific aim of establishing MIC values and comparing inhibitory activity across the bacterial panel.

A consolidated summary of the antibacterial studies reported for various plant parts and extracts of *M. ferrea*, compiled from the available literature records, is presented in Table 1. The reported work spans seed, root/stem bark, leaf, flower, flower bud, seed oil, fruit and

whole-plant preparations, evaluated against a wide range of Gram-positive and Gram-negative organisms using different extraction solvents and concentration ranges.

Table 1: Summary of previously reported antibacterial studies on different parts of *Mesua ferrea.*

S.No.	Plant part / extract	Gram-positive organisms tested	Gram-negative organisms tested	Reported effective concentration	Ref.
1	Seed (ethanol extract)	*S. aureus, Bacillus cereus*	*E. coli, Salmonella typhi*	5 mg/mL	[4]
2	Leaves (ethanol, methanol)	*Bacillus subtilis, S. aureus*	*E. coli, Pseudomonas aeruginosa*	1 µg/mL	[14]
3	Aerial parts – leaves & bark (water, methanol, ethanol, chloroform, ethyl acetate)	*Bacillus spp.*	—	5–30 mg/g dry wt	[16]
4	Flower buds (n-hexane, petroleum ether, ethyl acetate, acetone, ethanol, water)	*S. aureus*	*Pseudomonas aeruginosa*	0.015–1 mg/mL	[8]
5	Whole flower (methanol)	—	*Salmonella spp*. (5 strains, incl. ATCC 6539, NCTC 74)	5–400 µg/mL	[18]
6	Whole flower; leaves & fruit (methanol)	*S. aureus, Bacillus spp., Streptococcus pneumoniae, Sarcina lutea, Lactobacillus arabinosus*	*E. coli, Salmonella spp., Shigella spp., Klebsiella pneumoniae, Proteus mirabilis, Vibrio cholerae, Pseudomonas spp.*	5–200 µg/mL	[1]

2. MATERIALS AND METHODS

2.1 Plant material and authentication

Dried flower buds of *Mesua ferrea* were collected from Travancore, Kerala, India (8.6579° N, 77.3033° E) and authenticated by Dr S. Mutheeswaran, Xavier Research Foundation, Palayamkottai. A voucher specimen was deposited in the institutional herbarium for future reference (Register no. XCM-40645). The flowers were cleaned to remove extraneous matter, shade-dried, and pulverised into a coarse powder for further use.

2.2 Preparation of extract

The dried flower buds were coarsely powdered using a mixer grinder, passed through sieve no. 60, and stored in an airtight container until extraction. Fifty grams of powdered material was subjected to cold maceration with 300 mL of methanol. The mixture was shaken

thoroughly and allowed to stand at room temperature for five days with intermittent shaking, then filtered through muslin cloth followed by Whatman No. 1 filter paper. The clear filtrate was collected and stored in an airtight container under refrigeration until antibacterial evaluation.

2.3 Test microorganisms

Ten bacterial strains were evaluated: five Gram-positive organisms — *Staphylococcus aureus, Bacillus subtilis, Micrococcus lintus, Micrococcus luteus* and *Corynebacterium diphtheriae* — and five Gram-negative organisms — *Escherichia coli, Salmonella* Paratyphi, *Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Vibrio cholerae*. The corresponding culture collection numbers were NCIM 2065, NCIM 2501, NCIM 2707, NCIM 2200, MTCC 1738, NCIM 2079, NCIM 2063, NCIM 2018, NCIM 2169 and NCIM 2640, respectively (Table 2).

Table 2: Bacterial organisms included in the present study compared with organisms reported in other antibacterial studies on *M. ferrea*.

Category	Included in the present study	Reported in other antibacterial studies on *M. ferrea* (not evaluated here)
Gram-positive bacteria	*S. aureus*; *Bacillus subtilis*; *Micrococcus lintus*; *Micrococcus luteus*; *Corynebacterium diphtheriae*	*Bacillus cereus*; *Lactobacillus arabinosus*; *Corynebacterium* spp.; *Bacillus* spp.; *Streptococcus pneumoniae*; *Sarcina lutea*; *Enterococcus faecalis* (multiple isolates); *Enterococcus faecium*; *Streptococcus durans*; additional strain-specific *S. aureus* and *S. epidermidis* isolates; *S. saprophyticus*; *S. simulans*
Gram-negative bacteria	*E. coli*; *Salmonella* Paratyphi; *Klebsiella pneumoniae*; *Pseudomonas aeruginosa*; *Vibrio cholerae*	*Salmonella* Typhi; *Enterobacter aerogenes*; *Shigella* spp.; *Salmonella* spp. (multiple strains, incl. ATCC/NCTC isolates); *Proteus mirabilis*; *Pseudomonas* spp.; *Enterobacter cloacae*; *Serratia marcescens*; *Acinetobacter* spp.; *P. vulgaris*

2.4 Preparation of inoculum

Each bacterial strain was grown in nutrient broth at 37 °C for 18–24 h. The cultures were adjusted to the turbidity of a 0.5 McFarland standard, corresponding to approximately 1×10^8 CFU/mL, before use in the antibacterial assays.

2.5 Agar well diffusion assay

Antibacterial activity was evaluated by the agar well diffusion method. Nutrient agar plates were inoculated with 100 µL of bacterial suspension using a sterile spreader. Wells of 6 mm

diameter were prepared in the inoculated agar and 50 µL of the methanolic extract, at a concentration of 100 mg/mL, was added to each well. Methanol served as the negative control and a *ciprofloxacin 10 µg* served as the positive control. Plates were incubated at 37 °C for 24 h, and the zones of inhibition were measured in millimeters using a calibrated scale.

2.6 Minimum inhibitory concentration

The MIC was determined by broth microdilution using two-fold serial concentrations of the methanolic extract: 1000, 500, 250, 125, 62.5, 31.25, 15.63 and 7.81 µg/mL. Following inoculation with the standardised bacterial suspension, plates were incubated at 37 °C for 24 h. The MIC was defined as the lowest concentration at which no visible bacterial growth was observed.

2.7 Statistical analysis

The antibacterial activity was expressed as the zone of inhibition (mm), and the results were obtained from three independent determinations and expressed as mean ± standard deviation (SD). For each bacterial strain, differences among the methanolic *Mesua ferrea* extract, methanol negative control, and ciprofloxacin positive control were evaluated using one-way analysis of variance (ANOVA), followed by Dunnett's multiple-comparison test, with methanol considered as the reference control. A p value of <0.05 was considered statistically significant. Statistical analyses were performed using GraphPad Prism version 10.0.(Figure 2).

3. RESULTS

3.1 Bacterial strains

The study included ten bacterial strains, comprising five Gram-positive and five Gram-negative organisms. The culture collection identifiers are presented in Table 3.

3.2 Zone of inhibition

The methanolic extract produced measurable inhibition against nine of the ten tested organisms, with zones ranging from 16 to 28 mm. The largest zone was observed against *E. coli* (28 mm), followed by *S. aureus* (27 mm), while *K. pneumoniae*, *S.* Paratyphi, *V. cholerae*, *C. diphtheriae*, *P. aeruginosa* and the two *Micrococcus* strains showed zones between 16 and 22 mm. No inhibition was recorded for *B. subtilis*. The complete measurements are shown in Table 3 and the corresponding culture plates are shown in Figure 1.

Table 3: Zone of inhibition produced by the methanolic extract and the control treatments against the tested bacterial strains.

Organism	Extract, mean ± SD	Ciprofloxacin, mean ± SD
Escherichia coli	28.00 ± 1.00	26.00 ± 1.00
Salmonella paratyphi	22.00 ± 1.00	40.00 ± 1.00
Klebsiella pneumoniae	21.00 ± 1.00	35.00 ± 1.00
Pseudomonas aeruginosa	18.00 ± 1.00	31.00 ± 1.00
Vibrio cholerae	19.00 ± 1.00	35.00 ± 1.00
Staphylococcus aureus	27.00 ± 1.00	38.00 ± 1.00
Bacillus subtilis	0.00 ± 0.00	0.00 ± 0.00
Micrococcus lintus	18.00 ± 1.00	29.00 ± 1.00
Micrococcus luteus	16.00 ± 1.00	25.00 ± 1.00
Corynebacterium diphtheriae	19.00 ± 1.00	28.00 ± 1.00

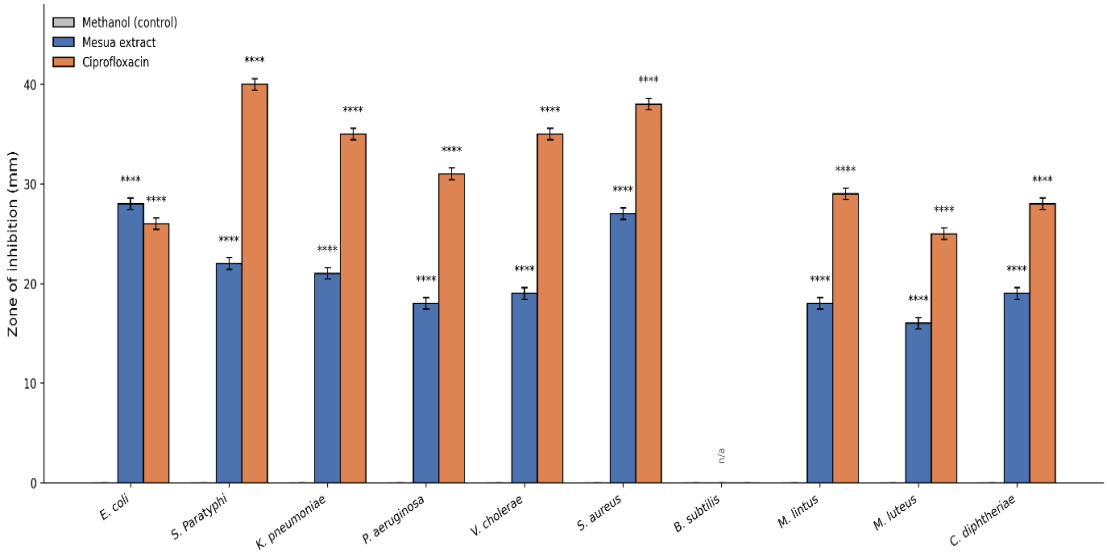

Figure 1: Dunnett's multiple-comparison analysis of the antibacterial activity of *Mesua ferrea* methanolic extract against selected bacterial strains.

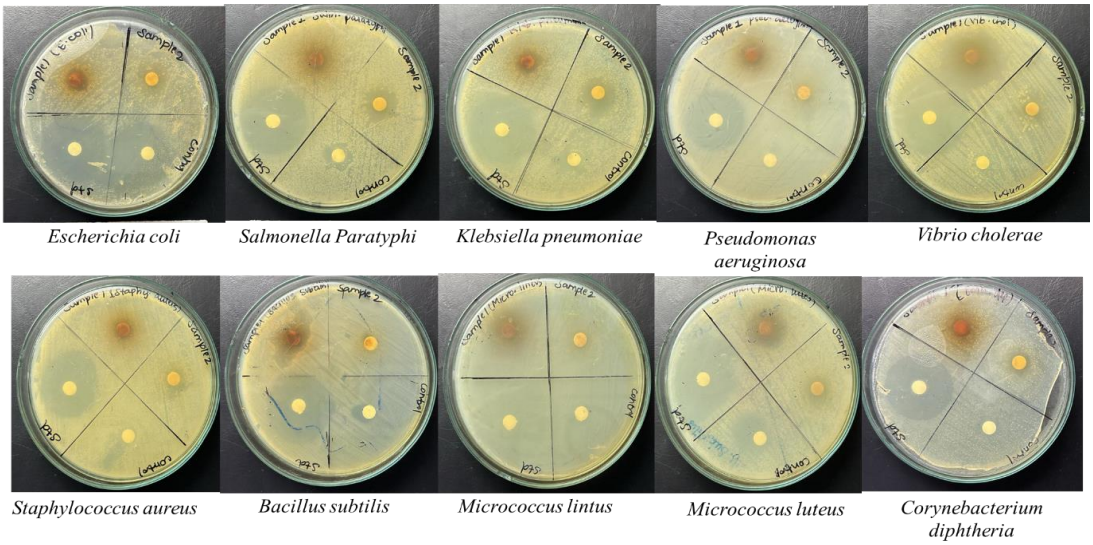

Figure 2: Zones of inhibition produced by the methanolic extract of *M. ferrea* flower buds (Sample 1/Sample 2), control and standard antibiotic (ciprofloxacin 10 µg), against the ten tested bacterial strains.

3.3 Minimum inhibitory concentration

The MIC endpoint was obtained from the two-fold dilution series described in Section 2.6. MIC values were 250 µg/mL for *E. coli*, *S.* Paratyphi, *K. pneumoniae*, *V. cholerae* and *B. subtilis*; 125 µg/mL for *S. aureus*, *M. lintus* and *C. diphtheriae*; and 62.5 µg/mL for *P. aeruginosa* and *M. luteus*. The observed MIC range was therefore 62.5–250 µg/mL, with *P. aeruginosa* and *M. luteus* showing the lowest MIC values among the organisms tested.

Table 4: Minimum inhibitory concentration of the methanolic extract of *M. ferrea* flower buds.

Bacterial strain	Gram reaction	MIC (µg/mL)	Interpretation
Escherichia coli	−	250	No visible growth at 250 µg/mL
Salmonella Paratyphi	−	250	No visible growth at 250 µg/mL
Klebsiella pneumoniae	−	250	No visible growth at 250 µg/mL
Pseudomonas aeruginosa	−	62.5	No visible growth at 62.5 µg/mL
Vibrio cholerae	−	250	No visible growth at 250 µg/mL
Staphylococcus aureus	+	125	No visible growth at 125 µg/mL
Bacillus subtilis	+	250	No visible growth at 250 µg/mL
Micrococcus lintus	+	125	No visible growth at 125 µg/mL
Micrococcus luteus	+	62.5	No visible growth at 62.5 µg/mL
Corynebacterium diphtheriae	+	125	No visible growth at 125 µg/mL

4. DISCUSSION

The present investigation demonstrated that the methanolic extract of *M. ferrea* flower buds exhibited antibacterial activity against a broad panel of organisms. In the agar well diffusion assay, measurable inhibition was observed against nine of the ten strains. *E. coli* and *S. aureus* showed the largest inhibition zones, whereas *B. subtilis* showed no detectable zone under the assay conditions used.

The MIC results provide a quantitative measure of inhibitory activity and showed a range of 62.5–250 µg/mL. *P. aeruginosa* and *M. luteus* exhibited the lowest MIC values (62.5 µg/mL), indicating greater susceptibility to the extract under the conditions tested. Several organisms showed an MIC of 125 µg/mL, while *E. coli*, *S.* Paratyphi, *K. pneumoniae*, *V. cholerae* and *B. subtilis* showed an MIC of 250 µg/mL.

The observed antibacterial activity is consistent with earlier reports describing the antimicrobial potential of *M. ferrea* preparations (Table 1). Organisms commonly reported as susceptible in the literature, particularly *S. aureus*, *E. coli* and *P. aeruginosa*, were also inhibited by the extract in the present study, supporting a degree of reproducibility across independent investigations. However, direct comparison among studies should be made cautiously, as antimicrobial activity can be influenced by plant material, geographical origin, extraction solvent, extraction conditions, extract concentration, bacterial inoculum and assay methodology.

As shown in Table 2, the present study evaluated only a subset of the organisms for which antibacterial activity of *M. ferrea* has been reported. Several clinically relevant organisms described in other studies — including *Enterococcus faecalis*, *Shigella* spp., *Proteus mirabilis* and additional *Salmonella* isolates — were not part of the present panel and represent candidates for future evaluation.

5. CONCLUSION

The methanolic extract of *Mesua ferrea* L. flower buds demonstrated *in vitro* antibacterial activity against nine of the ten tested bacterial strains in the agar well diffusion assay. MIC values ranged from 62.5 to 250 µg/mL, with *Pseudomonas aeruginosa* and *Micrococcus luteus* showing the lowest MIC values. These findings indicate that *M. ferrea* flower buds contain constituents with measurable antibacterial activity, broadly consistent with, while extending, the organism panel evaluated in earlier reports on this species. Further

investigation is warranted, including phytochemical characterisation, mechanism-oriented studies, testing against the additional organisms summarized in Table 2, standardised antimicrobial testing with replicate determinations.

Declarations

Ethics approval and consent to participate Not applicable — the study involved an *in vitro* bacterial assay and did not involve human participants, human data or animals.

Consent for publication Not applicable.

Availability of data and materials The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests The authors declare that they have no competing interests.

Funding This research received no external funding.

Authors' contributions A. Rajasekaran: conceptualization, supervision, manuscript review; B. Akshya: investigation, formal analysis, writing – original draft. Both authors read and approved the final manuscript.

ACKNOWLEDGEMENTS The authors express their sincere gratitude to the management of KMCH College of Pharmacy for providing the necessary facilities to carry out this work.

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